



# Measuring Fecal Contamination with Indicator Bacteria and Microbial Source Tracking

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# Overview

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- Background
- Fecal Indicator Bacteria
- Microbial Source  
Tracking
- Ongoing Science Center Work

# Background

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The Clean Water Act mandates states to identify impaired waters for designated pollutants and develop a Total Maximum Daily Load (TMDL) for pollutants over certain thresholds

A list of impaired streams requiring a TMDL is named a 303(d) list

# Background

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In Kentucky, numerous waterways are considered to be impaired because of fecal contamination and pathogens

Pathogens are responsible for numerous human diseases ranging from common diarrhea to hepatitis and pneumonia

# Background

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## Don't directly sample for pathogens

- A large number of pathogens exist
- Difficult to culture
- Highly infectious at very low doses
- Distributed sporadically in water

# Background

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## Fecal indicator bacteria used as proxy

- Can be easily cultured
- Have a correlation with pathogens
- Have a similar life-span as pathogens
- Examples: *Escherichia coli* (*E. coli*), total coliform, fecal coliform

# Fecal Indicator Bacteria

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## Fecal Indicator Bacteria concentration

- Filtration methods : CFU/ 100 mL
  - mTec *E. coli*
  - Modified mTec *E. coli*
- Enzyme substrate : MPN / 100 mL
  - Colilert *E. coli* / Total Coliforms
  - Colilert 18 *E. coli* / Total Coliforms





# Fecal Indicator Bacteria

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## Colilert analytical method (enzyme substrate)

- Water
  - 8-hour holding time
  - 24-hour incubation time 35 C°
  - Processed by USGS KYWSC
- Sediment
  - 24-hour holding time
  - 24-hour incubation time 35 C°
  - Samples chilled and immediately shipped to USGS Ohio Microbiology Laboratory

# Fecal Indicator Bacteria

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## Limitations

- Sources are unknown
- Different warm-bodied animals have dissimilar proportions of *E. coli* in their feces
- Most Fecal indicator bacteria are capable of propagating in water
- Fecal indicator bacteria can be found in bottom sediments

# Fecal Indicator Bacteria

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Knowledge of **both** host source (human and non-human) and geographic source of fecal contamination is critical for resource managers identify locations where remediation efforts will be most effective

# Microbial Source Tracking

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## Quantitative Polymerase Chain Reaction (qPCR) analytical method

- Based on concept that intestinal microbes between warm-blooded intestinal systems
- Current host-specific markers or assays
  - General **GenBac**
  - Humans **qHF183**
  - Ruminant **BoBac**
  - Dog **BacCan**

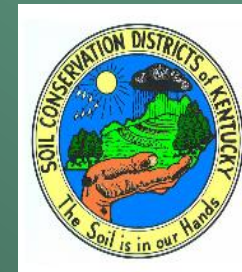
# Microbial Source Tracking

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## qPCR sample collection

- Collected by KY WSC staff
- Chilled and shipped immediately
- Samples processed by USGS Ohio Microbiology Laboratory

# Current Work – Little River



Local producers

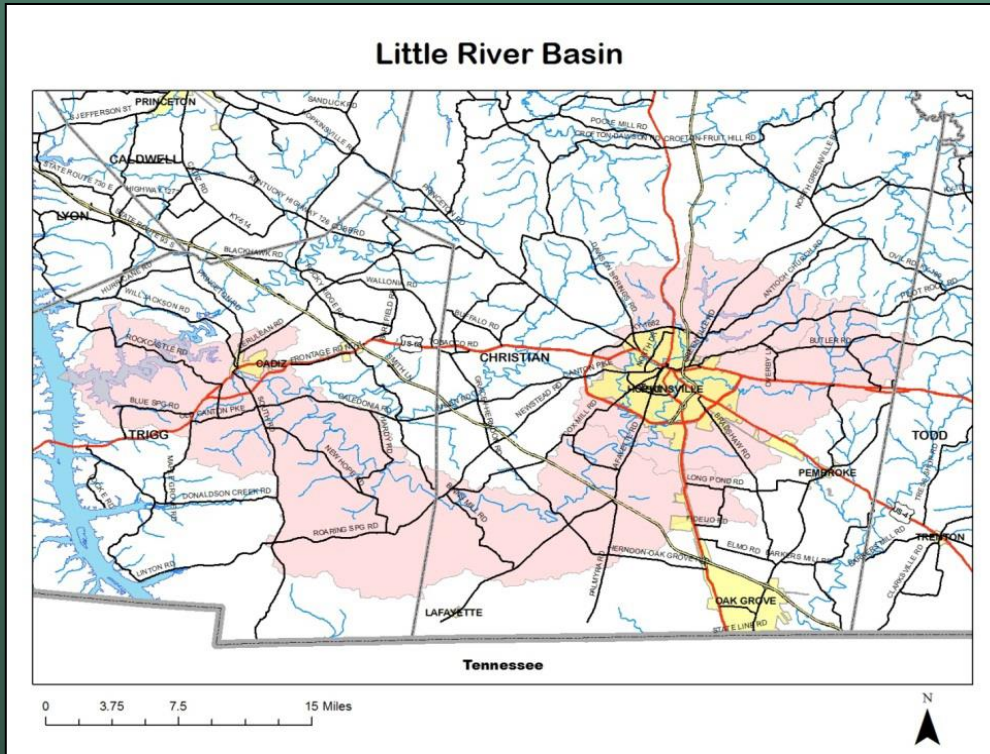
# Background

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In 2009, the Kentucky Division of Water completed and approved a pathogen TMDL for portions of the Little River Basin



# Background



Little River  
Basin: 600 mi<sup>2</sup>  
Counties

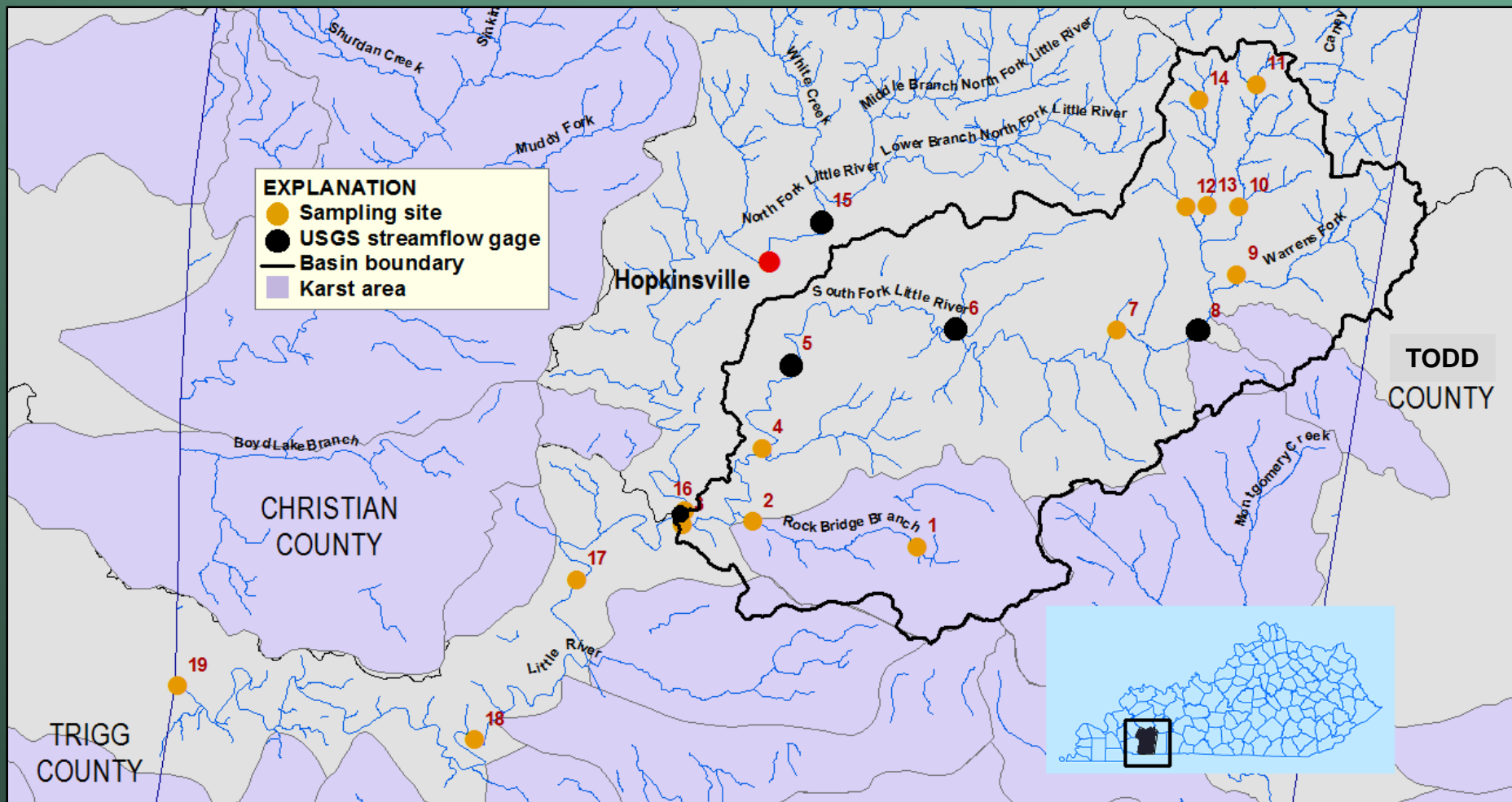
- Christian,  
Trigg, Todd,  
Mixed land-use



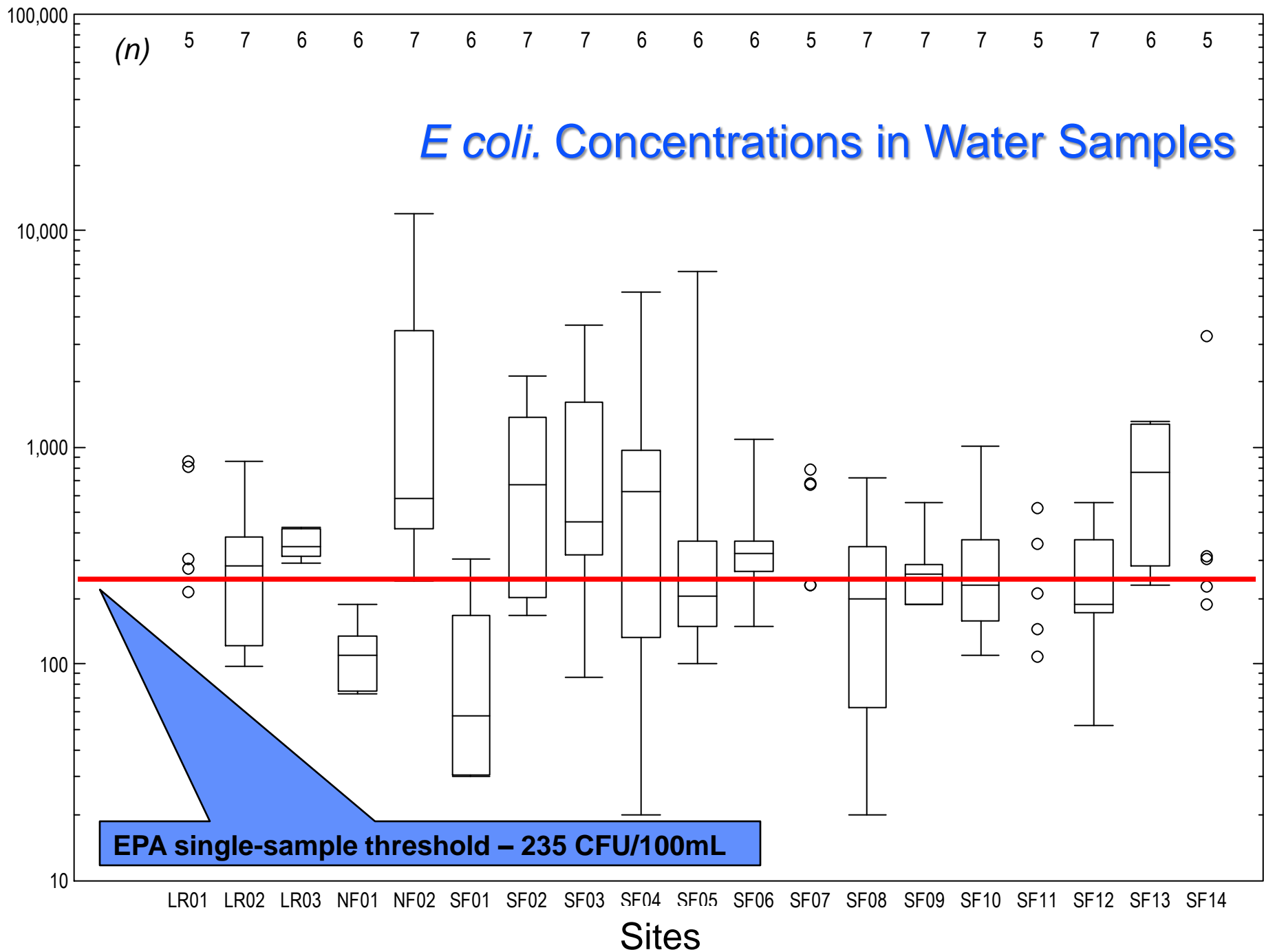
# Current Work – Little River

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Objective : To use microbial-source tracking and fecal-indicator bacteria to identify the sources and geographic distribution of fecal contamination in the South Fork of the Little River Basin



*E. coli*. Concentration MPN/100mL



# Current Work – Little River

SAMPLE DATE	SITE	General	Human	Canine	Ruminant
		----- copies/100 milliliters -----			
9/16/2013	NF02	22,000,000	470,000	7,700	37,000
9/17/2013	SF02	280,000	BDL	BDL	BDL
9/17/2013	SF06	310,000	BDL	17,000	BDL
9/17/2013	SF07	300,000	BDL	BDL	BDL
9/19/2013	SF10	170,000	BDL	BDL	BDL
9/17/2013	SF13	410,000	BDL	2,000	5,500
9/19/2013	SF14	270,000	BDL	BDL	BDL

BDL, Below Detection Limit

# Current Work – Little River

Date	Sample ID	General	Human	Canine	Ruminant
9/16/2013	WWTP 1	1.63E+09	1.15E+07	3.54E+05	3.08E+06
9/16/2013	WWTP 2	1.73E+09	8.68E+06	2.77E+05	1.69E+06
9/16/2013	WWTP 3	1.58E+09	1.02E+07	3.11E+05	1.66E+06
9/16/2013	WWTP 4	1.58E+09	8.17E+06	3.06E+05	1.84E+06
9/16/2013	WWTP 5	1.49E+09	1.02E+07	2.98E+05	1.32E+06

# Current Work – Little River

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- 2014 sample collection
- Data analysis and interpretation
- Report development
- Release of data to the public



# Questions?

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